The Haryana Veterinarian (December, 2024) **63(2),** 237-243 ISSN 0033-4359 (P) **Research Article**

COMPARATIVE HISTOCHEMICAL STUDY ON MALE AND FEMALE GOAT LIVER

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SUMMARY

The present study was conducted on 12 goat livers (6 male and 6 female). The tissues were collected from each lobe. The tissues were fixed and processed for paraffin sectioning for histochemical studies. The paraffin sections were stained with different histochemical stains for demonstration of neutral mucopolysacchrides, acid mucopolysacchrides, basic protein and glycogen. For cryosectioning fresh tissue were cut with cryostat microtome at -23º C and incubated in different substrates for histoenzymic localization of different phosphatases and oxidoreductases. The study revealed that reaction for neutral mucopolsaccharides and glycogen was more in centrilobular indicative of depletion of glycogen from periportal area during starvation. There was no difference in distribution of different histochemical moieties and localization of enzymes in different lobes in male and females. Within the lobule the activity of different enzymes was more mostly in periportal hepatocytes except GLD which was more in centrilobular hepatocytes. The activity of G-6-PD enzyme was comparatively more in females than male.

Keywords: Gender, Goat, Histochemical, Histoenzymic, Liver

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The liver is the body's largest organ and is next to brain in complexity. The parenchyma of liver comprised of hepatocytes which are supplied oxygen rich blood by hepatic artery and nutrition rich blood by portal vein. The sinusoids present between the hepatic cords receive blood from hepatic artery and portal vein and nourish surrounding hepatocytes. Thus, hepatocytes present closer to portal triad are getting blood rich in oxygen and nutrition as compared to hepatocytes near the central vein. This variation in oxygen and nutrition gradient forms the basis of heterogeneity in function of hepatocytes (Jungermann and Katz, 1989).Aside from typical physiological phenomena like enzyme activity, glycolysis, gluconeogenesis, metabolite distribution, and so on, pathological disorders have also been documented to have heterogeneity. The autoimmune hepatitis has been found to be more common in periportal hepatocytes (Jungermann and Katz, 1989) however fatty liver disease in the area towards central vein (Brunt, 2007). There have also been reports of gender-based differences in enzyme localization in male and female livers (Jungermann & Katz, 1989). In literature gender-based reports are available in rat & mice, guinea pig, human and pig but very few reports are available in domestic animals, so present study was planned to observe gender-based distribution of different histochemical moieties and enzymes in goat liver.

MATERIALS AND METHODS

The present study was conducted on 12 goat livers (6 male and 6 female) which were collected from local slaughter house, Ludhiana. The livers were thoroughly examined for any abnormal gross lesions. For histochemical study tissue samples from each lobe were collected and fixed in 10% neutral buffered formalin.The tissues were processed for paraffin sectioning and sections of 3-5 µm were cut with rotary microtome (Luna,1968). The paraffin sections were stained with Periodic acid Schiff, Alcian blue, Best carmine and Bromphenol blue stains for demonstration of neutral mucopolysaccharides, acid mucoolysaccharides, glycogen and total proteins (Luna 1968, Sheehan and Hrapchak, 1980). For histoenzymic localization of different phosphatases and oxidoreductases cryostat sections of 10 µm thickness of fresh unfixed tissues were cut at -23º C and incubated in different substrates as given in table 1. Total lipids were also demonstrated on cryostat sections (Chayen, 1969).

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RESULTS AND DISCUSSION

Histochemical observations

The histochemical distribution of neutral mucopolysaccharides, acid mucopolysaccharides, glycogen, basic proteins and total lipids, in different regions of male and female goat liver has been summarized in table 2. There were no differences observed in reaction of these moieties in different lobes of male and female liver.

Neutral mucopolysaccharides

The periportal hepatocytes, connective tissue, and bile duct in male and female goat liver showed weak to moderate reactions, whereas hepatocytes toward the central vein showed a strong reactivity in various lobes of male and female goat liver (Fig. 1). Bile duct, portal artery and portal vein also showed a variable weak to moderate reaction as observed by Modekar *et al.* (2003) who observed a weak PAS reaction in the portal blood arteries

in goat whereas Madhu (2013) reported a high PAS reaction in the apical boundary of columnar cells in the black buck's bile duct. The intensity of PAS reaction depends upon the degree of accumulation of carbohydrate component in the cell (Ramos *et al.*, 1980). The high activity in hepatocytes around central vein indicate more acuumulation of carbohydrates similar to the recordings of Singh (2019).

Acid mucopolysaccharides

Periportal and centrilobular hepatocytes showed a moderate reaction whereas connective tissue showed a moderate to strong reaction for acid mucopolysaccahride (Fig. 2). The bile duct showed negligible to weak reaction and hepatic artery and portal vein showed weak to moderate alcian blue reaction. The activity does not vary significantly between different lobes of liver and between male and female goat liver. The reaction in central vein was very weak. Aziz (1984) in sheep showed a moderate reaction to acid mucopolysaccharides in the major bile duct and central vein.

Basic protein

The hepatocytes in periportal and centrilobular areas were strongly positive for basic proteins whereas other components of male and female liver were weak to moderately positive (Fig. 3) similar to earlier findings of Gumucio and Miller (1981) in man and Singh (2019) in pig. The bromphenol blue method is a qualitative marker of basic proteins. So, a homogenous reaction of basic protein in all hepatocytes may conclude that all hepatocytes are involved in protein synthesis.

Glycogen

The periportal hepatocytes showed weak to moderate reaction for glycogen whereas hepatocytes around central vein were strongly positive (Fig. 4) and capsule, connective tissue, bile duct, hepatic artery, and portal vein showed negligible to weak reaction. So it was concluded that hepatocytes around central vein demonstrated intense reaction to glycogen deposition in the current study. The reaction varies insignificantly between different lobes of liver and between male and female goat liver. Although all hepatocytes are capable of producing and breaking down glycogen, periportal and centrilobular cells undergo these processes at varying times and rates. The type of diet and eating habits affect these processes during the 24 hour day/night cycle. In an earlier observation it was observed by Richard and Potter (1980) that when rats were fed a carbohydrate-rich diet the glycogen accumulation was more intense in hepatocytes in periportal area and after a long starving period the reaction of glycogen was more intense in hepatocytes around central vein. In present study the tissue samples were collected from local slaughter house where the animals were fasted for more than 24 hrs and result indicated that glycogen and PAS reaction was more in the hepatocytes around central vein. So, these results confirmed that during starvation the glycogen depletion starts first from hepatocytes in periportal and then proceeds towards centrivenous hepatocytes. Earlier Siddig *et al.* (2015) in camel and Rashad *et al.* (2017) in buffalo liver reported that different hepatocytes have different glycogen content.

Total lipid

The current study showed a weak reaction in capsule, connective tissue, central vein, bile duct whereas periportal hepatocytes, hepatic artery, and portal vein showed a variable weak to moderate activity (Fig. 5) which vary insignificantly between different lobes of liver and between male and female goat liver. According to Siddig *et al.* (2015), lipid droplets appeared to be widely distributed throughout the entire lobule with a propensity to concentrate in cells near the periphery as opposed to the centre. In the present study, the centrilobular hepatocytes showed a moderate reaction for total lipids in goat liver, which is in consistent with the results of Singh (2019) in pig liver.

Histoenzymology

The activity of different enzymes in different components of male and female livers has been summarized in table 3.

Phosphatases

Alkaline phosphatase (AKPase):

In present study, the periportal hepatocytes showed strong activity whereas hepatocytes near central vein showed moderate to strong activity (Fig. 6). The activity in male and female liver was almost same and no difference in activity was observed in different lobes of liver in male and female. Alkaline phosphatase enzyme is involved in transport activity and provides a barrier function as described by Meier-Ruge and Bruder (2008).The findings of present study are in consistent with findings of Singh (2019).

Glucose-6-phosphatase (G-6-Pase):

In the current study, it was found that the periportal hepatocytes exhibited strong G-6-Pase activity (Fig. 7). This may be because in the periportal zone, gluconeogenesis and glycogenolysis are catalysed, but in the centrilobular zone, glycolysis may be the main pathway of glucose synthesis. The activity in capsule, central vein, bile duct, hepatic artery, and portal vein was negligible to weak in both male and female goat liver and connective tissue

Table 1. Histoenzymic methods used on cryostat sections of goat liver

S.No.	Enzyme	Substrate	Method	Reference	Incubation time
A.	Phosphatases				
i)	Alkaline phosphatase (AKPase)	Napthol AS-MX phosphate disodium salt in combination with Fast Blue RR	Simultaneous coupling azo dye method using substituted naphthols	Barka and Anderson (1963)	30 minutes
ii)	Glucose 6- Phosphatase (G-6-Pase)	Glucose-6-phosphate and lead nitrate	Lead nitrate method	Barka and Anderson (1963)	20 minutes
B.	**Oxidoreductases and Esterases**				
i)	Glucose-6 phosphate dehdrogenase (G-6-PD)	Di-Na glucose-6-phosphate	-do-	-do-	30 minutes
ii)	Glutamic dehydrogenase (GLD)	Na-L glutamate	-do-	-do-	30 minutes
iii)	Lactic dehydrogenase (LDH)	Na-DL lactate	-do-	-do-	30 minutes
iv)	Malic dehydrogenase (MDH)	L-Malic acid	-do-	-do-	30 minutes
v)	Succinic dehydrogenase	Di-Na succinate	-do-	-do-	15 minutes
vi)	Nicotinamide adenine dinucleotide phosphate diphorase (NADPH-diphorase)	Co-enzyme (NADPH)	-do-	-do-	30 minutes
vii)	Reduced Nicotinamide adenine dinucleotide diphorase (NADH-diphorase)	Co-enzyme (NADH)	-do-	-do-	30 minutes
viii)	Non specific Esterases	Alpha napthol acetate	Napthol acetate method	Barka and Anderson (1963)	10 min

Table 2. Histochemical distribution of neutral mucopolysaccharides (NMPS), acid mucopolysaccharides (ACMS), basic proteins and lipids in goat liver

	Stroma								Parenchyma							
	Capsule		Connective tissue		Periportal hepatocyte		Centrilobular hepatocyte		Central vein		Bile duct		Hepatic artery		Portal vein	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
NMPS	+	+	+/++	+/++	+/++	+/++	+++	+++	0/+	0/+	+/++	+/++	++	++	+/++	+/++
ACMS	++	++	++/+++	++/+++	++	++	++	++	+	+	0/+	0/+	+/++	+/++	+/++	+/++
Glycogen	0/+	0/+	+	+	+/++	+/++	+++	+++	0/+	0/+	0/+	0/+	+	+	+	+
Basic Proteins	++	++	++	++	+++	+++	+++	+++	0/+	0/+	+	+	+/++	+/++	+/++	+/++
Total Lipids	+	+	+	+	+/++	+/++	++	++	+	+	+	+	+/++	+/++	+/++	+/++

0 negligible, + weak, ++ Moderate, +++ strong

showed weak activity, while the activity of the centrilobular hepatocyte was moderate as reported earlier by Gebharadt (1992), Macsween *et al.* (2002) and Singh (2019) in pig. Katz (1992) reported that the periportal hepatocytes are primarily involved in oxidative energy metabolism, gluconeogenesis, urea synthesis, bile production and protective metabolism which may be due to hepatic innervation zonal variations and periportal to perivenous gradients of hormones, metabolites and oxygen.

Non Specific Esterase (NSE)

Non-specific esterases are a group of enzymes associated with lipid metabolism. A negliglble to weak activity was demonstrated by capsule, connective tissue, central vein, bile duct, hepatic artery and portal vein, whereas periportal hepatocytes showed weak to moderate activity and centrilobular hepatocyte showed moderate activity of Non-specific esterases (NSE) in different lobes of liver in male and female (Fig. 8) as reported by Saigal *et*

Table 3. Histoenzymic localization of phosphatase, oxidoreductases and nonspecific esterases in goat liver

	Stroma								Parenchyma							
	Capsule		Connective tissue		Periportal hepatocyte		Centrilobular hepatocyte		Central vein		Bile duct		Hepatic artery		Portal vein	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
AKPase	0/+	0/+	+	+	+++	+++	++/+++	++/+++	+	+	++	++	++	++	+	+
G-6-Pase	0/+	0/+	+	+	+++	+++	++	++	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+
Non specific esterases	0/+	0/+	0/+	0/+	+/++	+/++	++	++	0/+	0/+	0/+	0/+	+	+	+	+
SDH	0/+	0/+	0/+	0/+	+++	+++	++	++	0/+	0/+	0/+	0/+	+	+	+	+
MDH	0/+	0/+	0/+	0/+	++/+++	++/+++	++	++	0/+	0/+	++	++	+	++	+	
LDH	0/+	0/+	0/+	0/+	++	++	+/++	+/++	0/+	0/+	+	+	+	+	+	+
GLD	0/+	0/+	0/+	0/+	+/++	+/++	+++	+++	0/+	0/+	+	+	+	+	+	+
NADH diaphorase	+	+	+	+	+++	+++	++/+++	++/+++	0/+	0/+	+	+	+	++	+	
NADPH diaphorase	+	+	+	+	+++	+++	++/+++	++/+++	0/+	0/+	+	+	+	+	+	+
G-6-PD	+	+	+	+	++	+++	++/+++	+++	0/+	0/+	+	+/++	+	+/++	+	+

0 negligible, + weak, ++ Moderate, +++ strong

al. (1992) in buffalo. The NSE activity suggested that these hepatocytes played a role in synthesis of lipids and phospholipids which are used for the formation of cell membranes.

Oxidoreductases

Oxidoreductases are the enzymes which are concerned with biological oxidation and reduction to carry out normal physiological functions.

Succinic Dehydrogenase (SDH)

SDH is a mitochondrial enzyme which helps in oxidative energy metabolism. The citric acid cycle and the respiratory chain are two important mitochondrial pathways that are necessary for oxidative phosphorylation and are regulated by succinate dehydrogenase. SDH is essential for the homeostasis of cells' reactive oxygen species (ROS), which include the production of superoxide and H_2O_2 as well as their removal (Tretter *et al.,* 2016). Succinic dehydrogenase (SDH) was shown to have a strong activity in the periportal hepatocytes of the liver but a moderate activity in the centrilobular hepatocyte. Capsule, central vein, bile duct and connective tissue showed negligible to weak succinic dehydrogenase (SDH) activity, whereas the hepatic artery, and portal vein displayed weak activity as reported earlier by Bhattacharya (1986) in goat liver. A strong activity of SDH has also been reported inperiportal region of rat liver (Racine-Samson *et al.*, 1996) and pig liver (Singh *et al.*, 2019).

Malic Dehydrogenase (MDH)

MDH is also a mitochondrial enzyme of oxidative energy metabolism. In the current study, the capsule, connective tissue and central vein showed negligible to weak reaction, bile duct showed moderate activity, whereas hepatic artery and portal vein all displayed low activity. Periportal hepatocytes showed moderate to strong activity, whereas centrilobular hepatocytes showed moderate activity. The activity varies insignificantly between different lobes of liver and between male and female goat liver. Similar findings were reported by Bhattacharya *et al.* (1987) in goats and Singh *et al.* (2019) in pig. MDH catalyses transformation of malate into oxaloacetate in the citric acid cycle (Aulbach and Amuzie, 2017) and this reaction is reversible.

Glucose-6-phosphate dehydrogenase

A key enzyme in the pentose phosphate pathway, G-6-PD is influenced by complex interactions between dietary components and hormones (Taniguchi *et al.*, 2016). The role of pentose phosphates in synthesis of nucleic acid has been documented in chicken (Fenneland Pearse, 1961). The G-6-PD activity was moderate in the periportal region and moderate to strong in the centrilobular region of male liver whereas strong activity of G-6-PD was seen in periportal and centrilobular region of female liver (Fig. 9). It was also concluded from the current study that female livers have higher activity of G-6-PD than male animals. This is true because female's livers are specially designed to convert carbohydrates into fat. Glucose-6-phosphate dehydrogenase was weak in the capsule and connective tissue, central vein, bile duct, hepatic artery and portal vein, similar to earlier findings of Singh *et al.* (2019).

Lactic dehydrogenase (LDH)

LDH is an enzyme involved in gluconeogenesis which

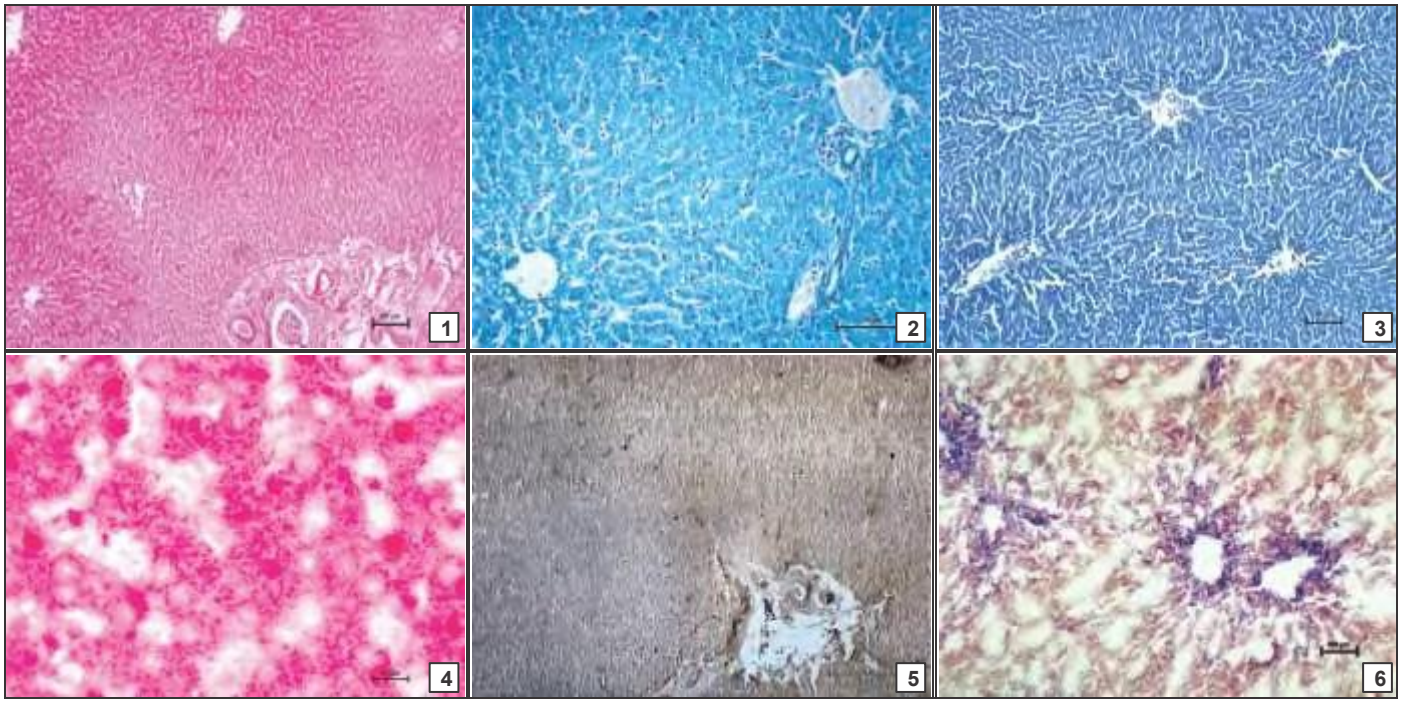

Fig. 1-6. **(1)** Liver of female goat showing PAS reaction in Periportal and Cenrilobular hepatocytes. PAS X 100; **(2)** Liver of male goat showing reaction for acid mucopolysacchride in PP and CL region. X 200; **(3)** Liver of female goat showing reaction for basic proteins in PP and CL region. Bromphenol blue X 200; **(4)** Liver of male goat showing Glycogen activity concentrated in Centrilobular region. Best Carmine X 1000; **(5)** Liver of female goat showing reaction for lipids. Sudan black B X 200; **(6)** Liver of male goat showing alkaline phosphatase activity in periportal region X100

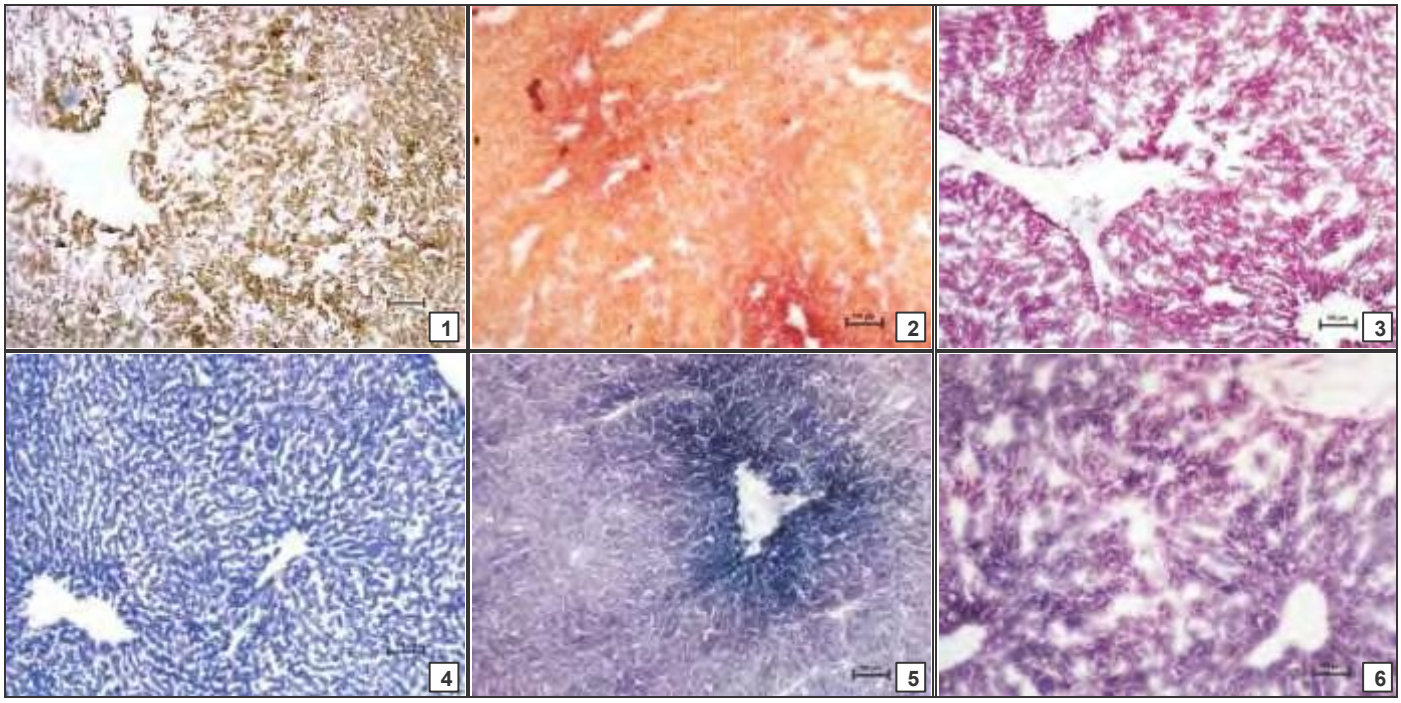

Fig. 7-12. **(7)** Liver of male goat showing G-6-Paseactivity. X 100; **(8)** Liver of male goat showing NSE activity. X 100; **(9)** Liver of female goat showing G-6-PD activity. X 100; **(10)** Liver of male goat showing NADH activity. X 100; **(11)** Liver of female goat showing GLD activity. X 100; **(12)** Liver of male goat showing NADPH activity. X 100

is an endergonic process so this enzyme helps in channelling alanine and lactate into gluconeogenic pathway. LDH reduces pyruvate into lactate and regenerates NAD from NADH (Lofgrenand Soderberg, 1998). A moderate activity of LDH was observed in periportal hepatocytes whereas capsule and connective tissue showed negligible to weak LDH activity. The centrilobular hepatocytes showed weak to moderate activity, central vein showed negligible to weak reaction whereas bile duct, hepatic artery and portal vein showed weak activity of Lactic

dehydrogenase (LDH) similar to the findings of Singh (2019). The activity did not vary significantly between various lobes and between male and female goat liver. The activity of LDH in periportal hepatocytes may contribute to the improvement of the liver's glycolytic pathway (Singh and Kumar, 2009).

Reduced Nicotinamide adenine dinucleotide diphorase (NADH-diphorase)

NAD+ is an essential component of cellular respiration, the chain of reactions that results in adenosine triphosphate (ATP) from the breakdown of nutrients. It does this by acting as a cofactor for oxidoreductases and dehydrogenases (Houtkooper *et al.*, 2010). NAD+ functions as an enzyme and is necessary for cellular metabolism and the creation of energy, as well as its reduced and phosphorylated forms, such as NADP+, NADH and NADPH (Ruggieri *et al.*, 2015). In the present study, periportal hepatocyte of the goat liver showed strong NADH activity and centrilobular hepatocytes showed moderate to strong reaction (Fig. 10). Capsule, connective tissue, bile duct, hepatic artery and portal vein showed weak activity of Reduced nicotinamide adenine dinucleotide diphorase (NADH) whereas central vein showed negligible to weak activity in both male and female goat liver, Whereas Bhattacharya *et al.* (1986), reported strong activity in the bile duct and moderate to strong activity in the portal veinbut strong activity in the periportal cells.

Glutamic dehydrogenase (GLD)

By deaminating glutamic acid, a Kreb's cycle intermediate, the GLD created alpha ketoglutaric acid. With the development of the GLD pathway in the Krebs cycle, the differential staining might be connected. Additionally, it aids with ammonia detoxification. The mitochondrial AST transforms the glutamate that GD produces into aspartate, which is used to synthesise urea. Additionally, glutamate is utilised in the mitochondrial synthesis of N-acetylglutamate, an allosteric activator of the enzyme carbamoyl phosphate synthase, which catalyses the initial step in the manufacture of urea (Ah Mew and Caldovic, 2011)Activity of glutamic dehydrogenase (GLD) was strong in centrilobular hepatocytes, negligible to weak activity in capsule, connective tissue and central vein, weak to moderate activity in periportal hepatocyte and weak activity was seen in bile duct, hepatic artery and portal vein (Fig. 11) in both male and female goat liver. Katz (1992) stated that hepatocytes in perivenous zone predominate in glutamine synthesis. It may be concluded that zonal alterations in activity of various enzymes may be due to difference in hepatic innervation and periportal to perivenous gradients of oxygen, hormones and metabolites and gene expression.

Reduced nicotinamide adenine dinucleotide phosphate diphorase (NADPH)

NADP (H)+, the phosphorylated form, takes part in anabolic processes such the production of fatty acids and cholesterol (Elhassan *et al.*, 2017). NADPH serves as a vital component of the body's defensive system by acting as a reducing radical in the face of oxidative stress. Glucose 6-phosphate dehydrogenase (G-6-PD) and malic enzyme are the main producers of NADPH (Taniguchi *et al.*, 2016). Capsule, connective tissue, bile duct, hepatic artery and portal vein showed weak activity, periportal hepatocytes showed strong activity, centrilobular hepatocytes showed moderate to strong activity and central vein showed negligible to weak activity of reduced nicotinamide adenine dinucleotide phosphate diphorase (NADPH) (Fig. 12). According to Bhattacharya *et al.* (1986). The endothelium, biliary epithelium and liver parenchyma showed a variable NADPH activity in goat liver.

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RETRACTION OF ARTICLE

This article earlier available at https://www.luvas.edu.in/haryana-veterinarian/download/harvet2016-dec/1.pdf entitled *"Occurrence of some organochlorine pesticide residues in poultry feed and meat"* has been retracted by the authors because of some error made during the data analysis process of the experimental observations due to counting the number of samples showing the concentration of pesticide below its corresponding Limit of Detection. All authors take full responsibility for this mistake and sincerely apologize for any inconvenience it may cause.

Editors